Examination of differences in added sugar consumption and urinary sugar excretion between post-menopausal women with healthy weight and post-menopausal women with obesity

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Introduction

Dietary added sugar is a commonly consumed nutrient, and a nutrient that is a part of many foods in the daily diet. According to the World Health Organization, added sugar (AS) intake should not exceed 10% of a person's total energy intake, and ideally, less than 5% of the diet consists of AS, to promote optimal health (World Health Organization, 2016). On average, AS makes up 13% (239 calories) of the average American woman's diet who is over the age of 20 (CDC, 2016). Added sugars are defined as those that are added during food processing and preparation (Tasevska, 2015). The wide availability of processed foods and the over consumption of these foods ultimately leads to this high consumption of AS (Dorton, 2017).

Self-reported intakes of AS are often underreported (Tasevska, 2014). Self-reported intake of AS contain inaccuracies due to many factors, and may be a source of error when assessing relationships between AS intake and disease outcomes. Self-reported intake data is best obtained from a weighed, 24-hour food record, with multiple days of intake (Freedman, 2017); this method of assessment is the "gold standard" for self-report. The accuracy of the estimations of dietary intake from food records may be enhanced by pairing self-reported data with a nutritional biomarker (Tasevska, 2015).

A urinary biomarker allows for nutrients in the urine to be analyzed objectively (Harpole, 2016), as the levels of these nutrients are measured directly from the urine that is excreted, following daily dietary consumption and does not require self- report. To better analyze AS intake, a biomarker, specifically a non-

invasive urinary biomarker, would be useful for a more accurate account of what is consumed. Currently, an enzymatic method of determining urinary sucrose and fructose is being investigated for its potential usefulness as a biomarker of added sugar intake (Tasevska, 2014).

Obesity related diseases in women are often correlated to a timeframe in life defined as menopause, a period of significant physiological changes that oftentimes lead to increased weight gain, and other complications (Siciliano, 2018). This new physiological point in a women's life, can lead to new eating habits to help cope with the bodily changes (Siciliano, 2018), and these habits can include increased AS consumption and this increased consumption can lead to a higher BMI and body weight (Siciliano, 2018). This higher body weight is attributed to many factors (Siciliano, 2018). The objective of my research project is to compare dietary intake, specifically added sugar consumption, in lean and obese postmenopausal women using measures of self-reported intake and compare urinary sucrose and fructose excretion in lean and obese women using an enzymatic assay.

Methods

The Added Sugar Biomarker Study (ASBS) used an experimental pre-post test, single group design to test the sensitivity of a urinary sucrose/fructose biomarker in detecting added sugar in the diet. Eligibility included being a postmenopausal female (natural amenorrhea greater than 12 months), 40 years of age or greater, a BMI between 18.5-24.9 kg/m^2 (lean group) or greater than $30kg/m^2$ (obese group), having a device capable of taking photographs and being able to take photos and send photos from this

device, and understanding English and able to write at the 8th grade level, with the ability to give written, informed consent to participate. Exclusion criteria from this study included a history of diabetes mellitus or prediabetes, renal disease, gastrointestinal disorders, or other conditions that could alter sucrose metabolism or excretion, pregnant or nursing women, and those unable to give informed consent. Women were recruited from the Franklin county area with the use of the website ResearchMatch.com as well as flyers posted around The Ohio State University Columbus campus. Recruitment of participants took place from June 2016- June 2017. For inclusion, a potentially eligible participant contacted the researcher who read her a study script and then orally asked questions for screening of eligibility. If the participant was potentially eligible from the results of the telephone screening, she was scheduled for an in person baseline screening where eligibility was confirmed and informed consent was obtained. If eligible, the researcher obtained anthropometric measurements, medical history, prescription drug history, a measure of normal physical activity level using the Godin survey (Godin, 1985), and demographic information.

At the baseline visit, the Registered Dietitian Nutritionist (RDN) explained to the participant how to complete a weighed, photographed food record, including making note of the time of intake, full detail of food consumed, including brand names if applicable, and gram weight of each food item. The participant was provided with a table to record this information and written instructions for completing the food record and collecting fasting urine, as well as measuring utensils and containers for weighing the foods. The RDN also instructed the participants on how to take and submit photos of the food consumed using a provided grid-mat to determine relative size of the food consumed.

Following the instructional baseline visit, two more visits were required for participation in the study. Both of these visits included the 24-hour weighed photographed food record, as detailed previously, and fasting urine collection. Within 7-10 days of the baseline-screening visit, the participant scheduled her first urine collection appointment at the clinic. At this appointment, the participants provided one weighed, photographed record of their normal daily intake over the previous 24 hours, and fasting urine was collected from the fast that began at midnight the morning of the clinic visit. First morning urine (collected at home) and a specimen collected in the clinic (termed "clinic urine") were obtained. After the first urine collection visit, the participant was provided with a sugar sweetened tea to consume during her second day of recording a 24-hour weighed, photographed food record. Between the first and second food record/urine collection visit, there was a one-week washout period where participants were instructed to consume their normal diets. On the day of their second food record, participants were asked to consume their normal diet plus the provided sugar-sweetened beverage. The following morning, women collected their second fasting urine sample and brought this to the Orchard lab for their final study visit where clinic urine was obtained and the RDN reviewed and collected the food record.

Urine was analyzed in a random order using assigned participant identification numbers. Each sample was thawed and filtered using 0.45 µl syringe filters prior to analysis. Each sample from each participant was analyzed on a single 96 well plate in triplicate. The participant samples from Day 1 and Day 2 first morning urine, clinic urine, and a pool sample from all urine samples from each day were analyzed for each participant. The urine samples were analyzed with an enzymatic Sucrose/D-Glucose/ D-

Fructose Assay; Boehringer Mannheim, R-Biopharm, Roche, Germany, which was modified to be run on a Microplate reader, in 96-well plates (Tasevska, 2014). Urinary creatinine was measured to normalize for hydration status (Luceri et al, 1996).

Results

Table 1 contains information regarding the characteristics of the study population. The mean body mass index (BMI) of the participants was 30.7±8.0, with an average age of 60 years old ±5 years, and 93% were white. The majority of the participants were married (60%) and one third of participants worked 40+ hours a week; almost half of the participants had an advanced degree. Almost three fourths of the participants had never smoked.

Table 1: Study Participant Characteristics

Characteristic	Mean±SD	N (% of total) n=30
Weight (lbs.)	179.1±47.0	
Height (in.)	64.2±2.0	
BMI (kg/m2)	30.7±8.0	
Age (years)	60.0±5.0	
Race Black White		 2 (6.7) 28 (93)
Ethnicity Not of Hispanic Origin		 30 (100.0)

Marital Status Single, never been married Married Divorced/Separated Widowed		 4 (13.3) 18 (60.0) 7 (23.3) 1 (3.3)
Employment Work 40+hours per week Work less than 40 hours per week Homemaker Retired Unemployed		 11 (33.7) 10 (33.3) 1 (3.3) 7 (23.3) 1 (3.3)
Education High School Diploma, GED Some college/ Associate's Degree Bachelor's Degree Advanced Degree		 1 (3.3) 8 (26.7) 7(23.3) 14 (46.7)
Smoke Never Past Current		 21 (70.0) 8 (26.7) 1 (3.3)
Total Leisure Activity Score	34.83±27	

Table 2 contains information about the dietary intake of the participants on both day 1 and day 2. For women with normal weight, there was a significant difference ($p=0.03$) between average energy (Kcal) intakes on day 1(1567±387kcal) compared to day 2 (1927±462kcal), with the higher intake day being day 2, when the sugar-sweetened beverage was consumed. Women with normal weight consumed a significantly greater amount of carbohydrates (235±70gm,$p<0.01$) total sugar (119±46gm, $p=0.01$) and added sugar (88±48gm, $p=<0.01$) on day 2, which is consistent with difference seen in calorie intake percentage from sugar consumption for day 2 as compared to day 1. For women with obesity, there was an increased carbohydrate (217±98g, $p=<0.01$), and added sugar consumption

(83±40g, p=<0.01) on day 2 . Between groups on day 1, there was a significant difference between percent of calories from protein (p=0.03), with women with obesity consuming a greater percentage of their calories as protein on day 1. Also on day 1, women with obesity consumed double the amount of sucrose, compared to the women with normal weight. On day 2, there were no significant differences in dietary intake between groups.

Table 2: Dietary Intake Characteristics

Dietary Variable	Women with normal weight (BMI 18.5-24.9)					Women with obesity (BMI > 30)						
	Day 1		Day 2			Day 1		Day 2				
	Mean (SD)	Range	Mean (SD)	Range	p^	Mean (SD)	Range	Mean (SD)	Range	p ^	p ^^	p ^^^
Energy (kcal)	1567±387	824-2525	1927±462	1300-2852	**0.03**	1664±555	976-2809	1669±440	1165-3173	0.98	0.60	0.14
Protein (g)	62±25	19-127	65±21	37-129	0.08	77±26	31-128	65±20	37-102	0.66	0.15	0.99
% kcal Protein	15±4	9.0-26	14±5	6.0-26	0.18	19±4	11.0-24	16±5	8.0-24	0.49	**0.03**	0.31
Fat (g)	61±32	34-163	84±35	50-160	0.72	69±32	22-154	64±25	28-108	0.22	0.53	0.10
% kcal Fat	33±10	17-56	37±9	23-60	0.07	36±12	20-71	34±12	16-60	0.78	0.48	0.46
Carbohydrate (g)	201±59	110-314	235±70	122-370	**<0.01**	193±88	63-395	217±98	111-497	**<0.01**	0.77	0.59
%kcal Carbohydrate	51±12	20-71	47±9	27-60	0.54	45±13	12.0-66	49±14	24-72	0.15	0.24	0.62
Total Sugar (g)	79±27	20-137	119±46	53-255	0.01	79±46	32-221	118±58	16-278	0.06	0.97	0.97
Added Sugars (g)	43±26	0-98	88±48	36-244	<0.01	38±30	1.0-95	83±40	1.0-182	<0.01	0.7	0.77
% kcal Added Sugar	12±8	0-28	18±7	7.0-34	0.03	9±6	0-22	20±7	0-33	<0.01	0.31	0.56
Sucrose (g)	16±9	13-271	82±48	11-232	0.36	33±23	4.0-75	74±29	3.0-135	0.42	**0.02**	0.59
Fructose (g)	16±9	13-271	10±8	1.0-35	0.25	18±12	1.0-42	16±17	1.0-65	0.69	0.73	0.25

^: Difference between Day 1 and Day 2 within groups

^^: Difference on Day 1 between groups

^^^: Difference on Day 2 between groups

Table 3 contains results of urinary sucrose and fructose analysis at all time points, the first morning fasting urine, the fasting clinic sample, and a pooled sample of both first morning and clinic samples. For study day 2, there was approximately three times the amount of sucrose detected in the urine of the women with normal weight than in the women with obesity for the fasting clinic sample of urine (p=0.043.) There were no significant differences between groups at other time points.

Table 3: Urine Analysis

Urinary Sugar (ug/mg Creatinine)	Study Day	Sample	Lean		Obese		P-value
			Mean ± SE	n	Mean ± SE	n	
Sucrose	1	First	116.4 ± 29.2	10	45.2 ± 25.6	13	0.0806
		Clinic	53.7 ± 24.7	10	38.5 ± 20.9	14	0.6432
		Pooled	100.9 ± 33.4	11	29.8 ± 30.7	13	0.1313
	2	First	80.0 ± 19.9	11	69.3 ± 18.3	13	0.6959
		Clinic	44.3 ± 10.0	9	14.2 ± 9.2	10	**0.0434**
		Pooled	53.8 ± 10.6	10	34.1 ± 9.3	13	0.1755
Fructose	1	First	16.4 ± 10.5	6	19.6 ± 8.6	9	0.8155
		Clinic	10.9 ± 7.2	7	14.9 ± 6.3	9	0.6819
		Pooled	16.0 ± 12.3	6	25.8 ± 10.7	8	0.5603
	2	First	16.2 ± 44.1	8	77.3 ± 41.6	9	0.3304
		Clinic	10.5 ± 4.1	8	12.2 ± 3.8	9	0.7692
		Pooled	11.3 ± 18.1	9	34.3 ± 16.4	11	0.3580

Discussion

Dietary components analyzed in parallel with urine have been a useful tool for measurement of nutrient intake, absorption and excretion from the body. In the ASBS, nutrient intake was documented using a weighed and photographed 24-hour food record and urine was analyzed for excretion of sucrose and fructose using an enzymatic assay. As expected, on day 2 of the study, the women reported consuming

more added sugar, a likely result of the sugar-sweetened beverage provided. Also on day 2, the urine collected from the participants indicated that there was significantly greater sucrose excretion in women within the normal weight range compared to women with obesity at the clinic visit.

In the ASBS study, we detected a higher amount of sucrose in the urine at the clinic visit on day 2 in normal weight women compared to women with obesity, but not a higher amount of fructose. In the Campbell study, there were 298 healthy women and 200 healthy men a 24-hour urine collection method was used, and the urine was analyzed using an enzymatic assay (Campbell, 2017). The Campbell study found no statistical difference between groups based on BMI, in sucrose or fructose excretion (Campbell, 2017), while in the ASBS, there was a significant difference between groups in the sucrose excretion, with women of normal weight excreting approximately triple the amount of sucrose compared to the women with obesity on study day 2. In the Campbell study, the participants were grouped based on age, those who were younger than 50 years old and those who were older than 50 years old (Campbell, 2017). In the ASBS, we only enrolled post-menopausal women and grouped participants based on weight; those with normal weight and those with obesity.

In a study conducted with healthy adult men and women, participants were asked to complete various forms of dietary assessment. When the healthy women completed dietary food records, there was a high correlation to what was reported in the food record, and what was then detected during spot urine collection (Kuhnle, 2015), using a similar enzymatic analysis to what was used in the ASBS. No

correlation analysis has been completed, but will be in the future. In the ASBS population, the women were healthy in that all women in the study had no prior diagnosis of diabetes, irritable bowel syndrome, or any other digestive disease. The women in the ASBS were of both normal, healthy weight, and with obesity. In the Kuhnle study, sources of added sugar intake were analyzed, and it was found that there was a correlation between the sucrose consumed and the sucrose excreted (Kuhnle, 2015). In the ASBS, AS consumption was not analyzed based on each source of AS. In future data analysis, analyzing the sources of the sugar may allow for a greater understanding of the results. By breaking down AS intake by source of sugar, it could potentially show which types of sugar, whether table sugar or high fructose corn syrup for example, would be better detected by a urinary enzymatic assay. Future research could add a clinical component where blood glucose, hemoglobin A1c, and other clinical tests would be run to help indicate if sucrose and fructose excretion may be modified by an undiagnosed health condition.

In conclusion, our results suggest that women of normal weight consume less sucrose in their usual diet when compared to women with obesity. The ASBS results do not indicate that there are significant differences in urinary sucrose excretion in women of normal weight versus women with obesity, except when consuming a sugar-sweetened beverage. When a sugar-sweetened beverage is consumed, our results suggest that women of normal weight excrete more sucrose. Future analysis will examine correlations of dietary intake with urinary sucrose and fructose excretion in this sample of postmenopausal women.

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